中華農業研究 28(1):45~49(1979)

# Diploidization of haploid rice plants by colchicine treatment [1]

Ming-hwa Lin [2]

**Abstract**: Haploid plants derived from anther culture of three rice (*Oryza sativa* L. ) varieties, Tainan 5, Taichung 65, and Taichung Yu 196, were treated with four different concentrations (0.05, 0.1, 0.25, and 0.5 %) of colchicine solution by leaf-sheath injection method. Diploid seeds were successfully obtained from the treated haploid plants. The percentage of plants producing diploid seeds and the percentage of tillers bearing diploid seeds was found to be 45 and 3.2%, respectively. The number of diploid seeds per tiller ranged from 5.3 to 6.4 with an average of 5.7, while the number of diploid seeds per plant ranged from 9.3 to 19.2 with an average of 12. It is suggested that 0.05 or 0.1% colchicine solution is suitable for chromosome doubling in haploid rice plants by leaf-sheath injection method.

Pure lines of diploid plants can be obtained directly by doubling the chromosomes of haploid plants. This method not only saves time but also eases the procedure of selection for desirable characters in plant breeding programs [8,11] Therefore, successful chromosomal doubling of haploid plants is one of the prerequisites for using haploidy in plant breeding. In rice (*Oryzas ativa* L. ) , it has been demonstrated that large numbers of haploid plants can be obtained from anther culture [1,2,3,9,14,15] . This paper reports an effective method for chromosomal doubling in anther-derived haploid of rice.

## MATERIALS AND METHODS

The haploid rice plants used in this study were derived from anther culture of the varieties, Tainan 5, Taichung 65, and Taichung Yu 196 [3] . Haploid plantlets of 10-15 cm in height were transplanted from the culture tubes to clay pots containing about 2 kg soil . When the plants developed 5-8 tillers, each was divided into four parts and each part was again transplanted to a larger clay pot containing 5 kg soil. One gram ammonium sulfate was applied as top-dressing two weeks after transplanting. The four pots of plants from a single callus were then treated with four different concentrations of colchicine solution, i. e. 0.05, 0.1, 0.25, and 0.5%, at the maximum tillering stage by leaf-sheath injection method.

The injection was done at 1.5-2.0 cm from the meristem of every tiller by using a

1. Contribution No. 866 from the Taiwan Agricultural Research Institute.
2. Agronomist, Department of Agronomy, TARI, Wufeng, Taichung, Taiwan 431, ROC.

2 ml-syringe. The amount of the solution injected was estimated by observing the solution exuding from the center unfolding leaf of the tiller. The injected haploid rice plants were allowed to grow to maturity. The number of the diploid spikelets per panicle and the number of tillers with diploid spikelets plant were also noted. The panicles with diploid spikelets were then covered with paper bags and allowed to grow to maturity.

## RESULTS AND DISCUSSION

The haploid rice plants showed growth disturbance one to two weeks after injection with colchicine. The central leaf of the tiller wilted and the whole plant was stunted. However, a large number of new tillers emerged three to four weeks later. Most of these new tillers grew normally and formed panicles. Usually, one or more diploid spikelets were produced in one panicle. Sometimes, more than one tiller produced diploid spikelets in a plant. Almost all diploid spikelets were fertile and developed seeds (Fig. 1) .

**Table 1.** The effects of diploidization in haploid rice plants by leaf-sheath injection method.

| Varieties | Conc. of colchicine (%) | No. of plants treated | No. of plants producing diploid seeds | %. of plants producing diploid seeds | %. of tillers producing diploid seeds | No. of seeds per tiller | No. of seeds per plant |
|---|---|---|---|---|---|---|---|
| Tainan 5 | 0.05 | 2 | 2 | 100 | 9.7 | 8.5 | 25.5 |
| | 0.10 | 2 | 2 | 100 | 8.1 | 8.8 | 2.02 |
| | 0.25 | 2 | 1 | 50 | 4.2 | 8.5 | 17.0 |
| | 0.50 | 2 | 0 | 0 | 0 | 0 | 0 |
| Taichung 65 | 0.05 | 10 | 6 | 60 | 2.9 | 3.1 | 4.7 |
| | 0.10 | 10 | 5 | 50 | 3.0 | 3.2 | 5.8 |
| | 0.25 | 10 | 3 | 30 | 1.6 | 2.4 | 4.0 |
| | 0.50 | 10 | 4 | 40 | 4.8 | 7.8 | 25.3 |
| Taichung Yu 196 | 0.05 | 3 | 0 | 0 | 0 | 0 | 0 |
| | 0.10 | 3 | 1 | 33.3 | 0.8 | 1.0 | 1.0 |
| | 0.25 | 3 | 1 | 33.3 | 2.4 | 13.0 | 26.0 |
| | 0.50 | 3 | 2 | 66.7 | 4.4 | 0.8 | 7.0 |
| Pooled | 0.05 | 15 | 8 | 53.3 | 3.2 | 5.3 | 9.9 |
| | 0.10 | 15 | 8 | 53.3 | 3.1 | 4.9 | 9.3 |
| | 0.25 | 15 | 5 | 33.3 | 2.0 | 6.1 | 11.0 |
| | 0.50 | 15 | 6 | 40.0 | 4.4 | 6.4 | 19.2 |
| | | 60 | 27 | 45.0 | 3.2 | 5.7 | 12.0 |

**Fig. 1.** The diploid seeds induced from haploid rice plants by leaf-sheath injection method.
a……few seeds derived from chromosome doubling.
b……fertile panicle derived from chromosome doubling.

As the pooled data shown in Table 1, 27 (45%) out of 60 haploid plants successfully produced diploid seeds and 3.2 percent of tillers bearing diploid seeds. The highest (19.2) and lowest (9.3) numbers of diploid seeds were found to be respectively from 0.5 and 0.1% colchicine treated plants. The similar trend was observed for the number of tillers producing diploid seeds.

Jensen suggested several methods for chromosome doubling in haploid plants [5]. Some of these have been proven to be very successful in tobacco [4,6,7]. In rice, the chromosome number of haploid plants could be doubled by irradiation [12,13] but its use is still limited by the availability of proper facilities and may also cause deleterious mutation. Woo and Su obtained 14 diploid seeds from one haploid rice plant treated with 0.05% colchicine [15]. However, their method of treatment was not clearly described. Since the meristem of rice plants is surrounded by leaf-sheath during the vegetative stage, it is difficult to use the conventional methods for colchicine treatment. The results of this study showed that 45% of the plants injected with colchicine produced one or more diploid seeds. The rate of success can be further improved by vegetative propagation of tiller separation or ratoon culture of a haploid plant before the treatment. Thus, the leaf-sheath injection method of colchicine treatment is very effective in doubling

the chromosomes of haploid rice plants.

It is expected that with the easy method devised in the present study, breeding for doubledhaploid rice varieties through haploid plants from anther culture would be promoted in the future.

**Acknowledgement**: The writer wishes to express his sincere gratitude to Dr. Sung-ching Hsieh, head of Agronomy Department, Taiwan Agricultural Research Institute, and Dr. Chi-chung Chen, Professor of Botany Department, National Taiwan University, for their comments and reading the manuscript.

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# 秋水仙精處理單倍體水稻之倍加[1]

林 明 華[2]


## 摘 要

利用臺南5號、臺中65號及臺中育196號等三個水稻品種或品系之花藥培養誘導來的單倍體植株，以四種不同濃度（0.05、0.1、0.25和0.5%）的秋水仙精進行葉鞘注射法處理，可成功地獲得二倍體種子。單倍體植株產生二倍體種子及產生二倍體種子的分蘗的百分率分別爲 45% 和3.2%。每分蘗的二倍體種子數在 5.3 到 6.4 粒之間，平均爲 5.7 粒，而每株的二倍體種子數在 9.3 到 19.2 粒之間，平均爲 12 粒。由試驗結果建議 0.05 和 0.1% 的秋水仙精濃度可爲葉鞘注射法處理單倍體水稻的染色體倍加之適宜濃度。


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1. 臺灣省農業試驗所 研究報告 第 866 號 。
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